Accepted Manuscript

Increased expression of *PLS3* correlates with better outcome in Sézary syndrome

S.E. Boonk, W.H. Zoutman, H. Putter, C. Ram-Wolff, M. Felcht, C.D. Klemke, A. Ranki, P. Quaglino, S. Whittaker, M. Bagot, R. Willemze, M.H. Vermeer

PII: S0022-202X(16)32609-4

DOI: 10.1016/j.jid.2016.10.025

Reference: JID 591

To appear in: *The Journal of Investigative Dermatology*

Received Date: 29 April 2016

Revised Date: 12 October 2016

Accepted Date: 12 October 2016

Please cite this article as: Boonk SE, Zoutman WH, Putter H, Ram-Wolff C, Felcht M, Klemke CD, Ranki A, Quaglino P, Whittaker S, Bagot M, Willemze R, Vermeer MH, Increased expression of *PLS3* correlates with better outcome in Sézary syndrome, *The Journal of Investigative Dermatology* (2016), doi: 10.1016/j.jid.2016.10.025.

Increased expression of *PLS3* correlates with better outcome in Sézary syndrome

S.E. Boonk[1], W.H. Zoutman[1], H. Putter[2], C. Ram-Wolff[3-5], M. Felcht[6], C.D. Klemke[6,7], A. Ranki[8], P. Quaglino[9], S. Whittaker[10], M. Bagot[3-5], R. Willemze[1], M.H. Vermeer[1]

[1] Department of Dermatology, Leiden University Medical Center, Leiden, The Netherlands

[2] Department of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands

[3] INSERM U976, Hospital Saint-Louis, Paris, France

[4] Paris Diderot University, Hospital Saint-Louis, Paris, France

[5] Department of Dermatology, Hospital Saint-Louis, Paris, France

[6] Department of Dermatology, Venereology and Allergy, University Medical Center Mannheim, Ruprecht-Karls-University of Heidelberg, Mannheim, Germany

[7] Hautklinik, Städtisches Klinikum Karlsruhe, Karlsruhe, Germany

[8] Department of Dermatology and Allergology, University of Helsinki and Skin and Allergy Hospital, Helsinki University Central Hospital, Helsinki, Finland

[9] Department of Medical Sciences, Dermatologic Clinic, Turin University, Turin, Italy

[10] St. John's Institute of Dermatology, Division of Genetics and Molecular Medicine, Faculty of Life Sciences & Medicine, King's College London, London, United Kingdom

Corresponding author:

Stéphanie E. Boonk, MD

Department of Dermatology, Leiden University Medical Center

Albinusdreef 2 2300 RC Leiden, Netherlands

Telephone: 0031715262630

Fax number: 0031715248106

E-mail: s.e.boonk@lumc.nl

Manuscript word count: 999

Manuscript table count: 1

Manuscript figure count: 1

Reference count: 15

Supplementary files: 1

Abbreviations:

SS = Sézary syndrome

OS = overall survival

MF = mycosis fungoides

WHO = World Health Organization

EORTC = European Organization for Research and Treatment of Cancer

DSS = disease-specific survival

To the editor:

Increased expression of *PLS3* correlates with better outcome in Sézary syndrome

Patients with Sézary syndrome (SS), a rare erythrodermic and leukemic form of cutaneous T cell lymphoma, have a poor prognosis with a 5-year overall survival (OS) of 20-42% and a median OS between 2.5 and 5 years (Bernengo et al., 1998;Diamandidou et al., 1999;Kim et al., 2003;Agar et al., 2010;Talpur et al., 2012;Kubica et al., 2012).

Prognostic factors associated with a worse survival reported in SS include advanced age (Diamandidou et al., 1999;Kim et al., 2003;Foulc et al., 2003;Agar et al., 2010;Talpur et al., 2012;Kubica et al., 2012), short duration of skin lesions before diagnosis of SS (Foulc et al., 2003), previous history of mycosis fungoides (MF) (Bernengo et al., 1998;Kubica et al., 2012), elevated serum lactate dehydrogenase levels (Bernengo et al., 1998;Diamandidou et al., 1999;Foulc et al., 2003;Agar et al., 2010;Talpur et al., 2012;Kubica et al., 2012), (the degree of) nodal involvement (Diamandidou et al., 1999;Kim et al., 2003), and factors reflecting blood tumor burden, such as increased leukocyte counts (Bernengo et al., 1998;Vidulich et al., 2009;Talpur et al., 2012) or high Sézary cell counts (Bernengo et al., 1998).

However, the results of these studies are not consistent, which may be due to different diagnostic criteria of SS, such as inclusion of patients without a T-cell clone in the peripheral blood, and analysis of mixed populations of patients with SS and erythrodermic MF.

Recently we investigated the diagnostic significance of a large number of immunophenotypic and molecular biomarkers for SS in a group of Sézary patients (Boonk et al., 2016) that fulfilled the diagnostic criteria of the World Health Organization - European Organization for Research

and Treatment of Cancer (WHO-EORTC) classification (Willemze et al., 2005). None of these patients had a history of MF. Molecular biomarkers diagnostic for SS were copy number alterations in *MYC* (gain) and/ or *MNT* (loss), increased expression of *DNM3*, *TWIST1*, *EPHA4* and *PLS3* and decreased expression of *STAT4*.

Here we investigated the prognostic significance of these molecular biomarkers and previously reported clinical prognostic markers using the same cohort of Sézary patients. Between September 2009 and October 2013 sixty-four SS patients from six EORTC centers, including Helsinki (Finland), London (England), Leiden (Netherlands), Mannheim (Germany), Turin (Italy) and Paris (France) were included and followed until 30 January 2015. At inclusion of the study, clinical variables (gender, age at diagnosis, duration of skin lesions before diagnosis SS, lymph node involvement, leukocyte count, absolute CD4 count and Sézary cell count) were recorded and peripheral blood samples were collected for copy number variation and gene expression qPCR analysis, as described previously (Boonk et al., 2016). Lymph node involvement was defined by presence of enlarged lymph nodes of 1.5 cm or larger in the longest transverse diameter on computed tomography scan or histologically confirmed lymph node involvement.

Aberrant gene expression in the SS samples was compared to samples from patients with erythrodermic inflammatory dermatoses (EID) and healthy controls. ROC curve analysis was used to determine fixed cut-off thresholds for each individual gene expression qPCR assay with a specificity of 100% and an accuracy above 0.80. An one-tailed Mann-Whitney test was applied to test for significant differential expression between the SS and EID samples. *P*-values below 0.05 were regarded as statistically significant. The results of aberrant expression of genes *DNM3*,

TWIST1, *EPHA4*, *PLS3* and *STAT4* were included in the statistical analysis. A more detailed method section including these thresholds is added in the **supplementary material**.

Survival was calculated with the Kaplan-Meier method from the date of diagnosis until the patient's death or date of last follow-up. The median follow-up time after diagnosis was 45 months (range, 1-129 months). Twenty seven patients died during follow-up, including 21 SS related deaths. The disease-specific survival (DSS) after 1, 2, 3 and 5 years was 89%, 82%, 76% and 59%, respectively, and OS was 86%, 79%, 72% and 49%, respectively.

Univariate analysis of parameters with possible prognostic significance for DSS and OS was performed using Cox proportional hazards regression analysis and parameters that were significant at the 0.1 level were included in a multivariate analysis model. P-values below 0.05 were regarded as statistically significant.

Both in univariate and multivariate analysis upregulation of *PLS3* was associated with a significantly better outcome for DSS and OS (multivariate $P = 0.006$ and $P = 0.002$, respectively). Patients with upregulation of *PLS3* had a median survival of 71 months (range, 9-129) compared to only 33 months (range, 1-72) in SS patients with normal expression of *PLS3* (**Figure 1**). Upregulation of *DNM3* and *TWIST1* were associated with a better OS in univariate analysis ($P = 0.008$ and $P = 0.043$, respectively), but not in multivariate analysis ($P = 0.658$ and $P = 0.342$, respectively). Gain of *MYC*, loss of *MNT*, upregulation of *EPHA4* and downregulation of *STAT4* showed no association with DSS and OS (**Table 1**).

Of the clinical parameters both univariate and multivariate analysis showed that leukocyte count was a significant prognostic factor for DSS and OS (multivariate $P = 0.005$ and $P = 0.005$, respectively), while gender, age, duration of skin lesions before diagnosis, lymph node involvement, absolute CD4 count and Sézary cell count were not (**Table 1**).

PLS3 (T-plastin) is an actin-binding protein that is expressed in all normal cells of solid tissues that have a replicative role, but is normally not expressed in T cells (Lin et al., 1999). Expression of *PLS3* has been described as a specific marker of Sézary cells (Nebozhyn et al., 2006;Jones et al., 2012). Studies investigating the mechanism underlying dysregulation of *PLS3* expression in SS cells found no evidence for *PLS3* mutations within coding or promoter regions, but demonstrated significant hypomethylation of CpG dinucleotides 95-99 within the *PLS3* CpG island which was restricted to the *PLS3*+ cells (Jones et al., 2012). Reanalysis of recently published DNA methylation profiles (van Doorn et al., 2016) from 9 Sézary patients and 4 healthy controls included in this study confirmed this correlation between DNA methylation and *PLS3* expression (data not shown). A recent study found that constitutive *PLS3* expression was associated with apoptotic resistance to etoposide and suggested a role for cell survival in SS (Begue et al., 2012). How T-plastin expression is linked to a better outcome in Sézary patients is not known and should be the subject of further study.

Although for a rare disease as SS the number of included patients is relatively high, a limitation of this study is a small sample size yielding wide confidence intervals and these observations should be confirmed in an independent study.

In conclusion, we show that upregulation of *PLS3* is associated with a favorable disease outcome in patients with SS and that increased leukocyte count is a significant adverse prognostic factor for survival.

Conflict of interest: M.F. received travel grants from TEVA.

Acknowledgements: This study was supported by research funding from the Dutch Cancer Society to M.H.Vermeer, the Helsinki University Central Hospital Research Funds grant TYH2012232 to A. Ranki, the INCa-DGOS-Inserm grant to M. Bagot and the grants from the German Research Council (GRK2099 “Hallmarks of Skin Cancer” and FE 1282/2-1) to M. Felcht. In addition, this research was supported by grants from the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' National Health Service (NHS) Foundation Trust and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

References

Agar NS, Wedgeworth E, Crichton S, Mitchell TJ, Cox M, Ferreira S, et al. Survival outcomes and prognostic factors in mycosis fungoides/Sezary syndrome: validation of the revised International Society for Cutaneous Lymphomas/European Organisation for Research and Treatment of Cancer staging proposal. J Clin Oncol 2010;28:4730-39.

Begue E, Jean-Louis F, Bagot M, Jauliac S, Cayuela JM, Laroche L, et al. Inducible expression and pathophysiologic functions of T-plastin in cutaneous T-cell lymphoma. Blood 2012;120:143-54.

Bernengo MG, Quaglino P, Novelli M, Cappello N, Doveil GC, Lisa F, et al. Prognostic factors in Sezary syndrome: a multivariate analysis of clinical, haematological and immunological features. Ann Oncol 1998;9:857-63.

Boonk SE, Zoutman WH, Marie-Cardine A, van der Fits L, Out-Luiting JJ, Mitchell TJ, et al. Evaluation of immunophenotypic and molecular biomarkers for Sezary syndrome using standard operating procedures: a multicenter study of 59 patients. J Invest Dermatol 2016;136:1364-72.

Diamandidou E, Colome M, Fayad L, Duvic M, Kurzrock R. Prognostic factor analysis in mycosis fungoides/Sezary syndrome. J Am Acad Dermatol 1999;40:914-24.

Foulc P, N'Guyen JM, Dreno B. Prognostic factors in Sezary syndrome: a study of 28 patients. Br J Dermatol 2003;149:1152-58.

Jones CL, Ferreira S, McKenzie RC, Tosi I, Caesar JA, Bagot M, et al. Regulation of T-plastin expression by promoter hypomethylation in primary cutaneous T-cell lymphoma. J Invest Dermatol 2012;132:2042-49.

Kim YH, Liu HL, Mraz-Gernhard S, Varghese A, Hoppe RT. Long-term outcome of 525 patients with mycosis fungoides and Sezary syndrome: clinical prognostic factors and risk for disease progression. Arch Dermatol 2003;139:857-66.

Kubica AW, Davis MD, Weaver AL, Killian JM, Pittelkow MR. Sezary syndrome: a study of 176 patients at Mayo Clinic. J Am Acad Dermatol 2012;67:1189-99.

Lin CS, Lau A, Huynh T, Lue TF. Differential regulation of human T-plastin gene in leukocytes and non-leukocytes: identification of the promoter, enhancer, and CpG island. DNA Cell Biol 1999;18:27-37.

Nebozhyn M, Loboda A, Kari L, Rook AH, Vonderheid EC, Lessin S, et al. Quantitative PCR on 5 genes reliably identifies CTCL patients with 5% to 99% circulating tumor cells with 90% accuracy. Blood 2006;107:3189-96.

Talpur R, Singh L, Daulat S, Liu P, Seyfer S, Trynosky T, et al. Long-term outcomes of 1,263 patients with mycosis fungoides and Sezary syndrome from 1982 to 2009. Clin Cancer Res 2012;18:5051-60.

van Doorn R, Slieker RC, Boonk SE, Zoutman WH, Goeman JJ, Bagot M, et al. Epigenomic analysis of Sézary syndrome defines patterns of aberrant DNA methylation and identifies diagnostic markers. J Invest Dermatol, in press.

Vidulich KA, Talpur R, Bassett RL, Duvic M. Overall survival in erythrodermic cutaneous T-cell lymphoma: an analysis of prognostic factors in a cohort of patients with erythrodermic cutaneous T-cell lymphoma. Int J Dermatol 2009;48:243-52.

Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, et al. WHO-EORTC classification for cutaneous lymphomas. Blood 2005;105:3768-85.

Table:

Table 1. Results of univariate and multivariate analysis for variables at Sézary syndrome diagnosis. Parameters significant at the 0.1 level were included in multivariate analysis.

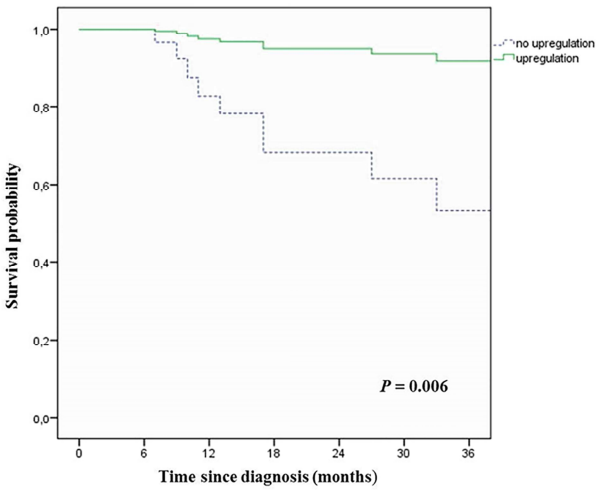
Variables	Median survival (months)	Univariate analysis DSS HR (95% CI)	*P*-value	Multivariate analysis DSS HR (95% CI)	*P*-value	Univariate analysis OS HR (95% CI)	*P*-value	Multivariate analysis OS HR (95% CI)	*P*-value
Gain in copy number of *MYC*			0.843				0.366		
Yes n=21	72 (1-129)	0.90 (0.32-2.54)				0.66 (0.26-1.63)			
No n=31	49 (7-86)	1				1			
Loss in copy number of *MNT*			0.355				0.388		
Yes n=35	68 (1-129)	0.61 (0.22-1.74)				0.68 (0.28-1.65)			
No n=17	49 (7-80)	1				1			
Upregulation of *DNM3*			0.337				0.008		0.658
Yes n=36	71 (9-129)	0.56 (0.17-1.83)				0.29 (0.12-0.72)		0.74 (0.20-2.75)	
No n=13	33 (1-72)	1				1		1	
Upregulation of *TWIST1*			0.197				0.043		0.342
Yes n=32	71 (1-129)	0.48 (0.16-1.46)				0.39 (0.16-0.97)		0.57 (0.18-1.81)	
No n=17	31 (7-80)	1				1		1	
Upregulation of *EPHA4*			0.312				0.311		
Yes n=32	49 (7-129)	1.95 (0.53-7.12)				1.70 (0.61-4.74)			
No n=17	68 (1-80)	1				1			
Upregulation of *PLS3*			0.027		0.006		0.001		0.002
Yes n=32	71 (9-129)	0.29 (0.10-0.87)		0.14 (0.03-0.56)		0.19 (0.07-0.49)		0.12 (0.03-0.46)	

No n=17	33 (1-72)	1		1		1		1	
Downregulation of *STAT4*			0.356				0.250		
Yes n=44	68 (1-129)	#				3.28 (0.43-24.77)			
No n=5	Not reached	1				1			
Gender			0.665				0.830		
Female n=25	49 (1-129)	1.21 (0.51-2.85)				0.92 (0.43-1.98)			
Male n=39	68 (1-115)	1				1			
Age at SS diagnosis (y) n=64		1.01 (0.96-1.05)	0.827			1.00 (0.97-1.04)	0.850		
Duration skin lesions n=60		0.98 (0.96-1.00)	0.075	0.99 (0.97-1.02)	0.447	0.99 (0.97-1.00)	0.152		
Lymph node involvement			0.356				0.420		
Yes n=18	49 (1-74)	1.82 (0.51-6.53)				1.55 (0.53-4.52)			
No n=23	Not reached	1				1			
Leukocyte count n=61		1.04 (1.01-1.06)	0.007	1.06 (1.02-1.10)	0.005	1.03 (1.01-1.06)	0.011	1.05 (1.01-1.08)	0.005
Absolute CD4 count n=59		1	0.146			1	0.265		
Sézary cell count n=47		1	0.123			1	0.204		

Figure legend:

Figure 1. Survival curves. Disease-specific survival (**a**) and overall survival curve (**b**) according the groups with and without upregulation of *PLS3*.

a.



b.